Motor Proteins

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1 Introduction

Interest in protein motors started with attempts to understand the operation of muscles. It was found that muscular contraction is driven by a class of proteins known as myosin. Biophysical understanding of this process necessitated postulating different intermediate states for this protein during contraction[?]. With the advent of increasingly sophisticated experimental techniques, emphasis was placed on the understanding of these proteins at a structural level. Other protein motors which are crucial for cellular activity were also identified. Now it is established that a significant part of the eucaryotic cellular traffic relies on motor proteins that move in a deterministic way along filaments with energy derived from hydrolysis of ATP. Three different families of motor proteins have been identified: kinesins and dyneins which move along tubulin filaments and myosin which move along actin filaments[?]. Another special class is the ATP synthase motors which manufactures ATP from ADP in the body by using energy derived from a transmembrane protonmotive gradient [?].

Physicists are attracted to motor proteins since they represent some of the smallest motors in existence operating at very high efficiency.(The kinesin motors operate at 40-50% efficiency and the ATP synthase operate at almost 100% efficiency). Explaining the physical mechanism of energy transduction and force generation in terms of the simplest model of protein is their usual goal[?].

The present understanding of these motor proteins and their operation can be summarized as follows[?]. The motor proteins usually have two globular heads which can bind to the filamental track joined together by a neck region. Crystal structures of actin and the motor domains of myosin and kinesin have been determined. The catalytic ATP-binding core of kinesins and myosin have surprisingly similar secondary structure even though there is little sequence homology. See figures for structural details. Filamentous actin and microtubules, are tracks which guide and direct the translocation motions. The mechanism by which the motors generate movement is poorly understood. It is thought that the hydrolysis of ATP and released energy elicit small changes in the protein structure surrounding the nucleotide, which then propagates to other regions of the protein. Ultimately these events become amplified into larger domain motions that are responsible for producing force and unidirectional motion.

In this paper the bio-chemical and physical approaches to the understanding of the operation of motor proteins is reviewed. It is both interesting and instructive to see the differences between the two approaches, the techniques used and the definition of understanding by the two approaches. Bio-chemical approaches are driven by the experimental methods and some of the experimental techniques used are reviewed. The physical approch is more theoretical and the major step is identifying a simple model and applying the theory of Non-equilibrium thermodynamics to the model.

2 Methods

The main emphasis of biologists has been to understand the mechanism responsible for biological motion in terms of structural changes. Various experimental techniques have been applied to study this and the goal is to identify all the intermediate conformational states during one ATP cycle.

Some experimental techniques used to study muscle proteins are[?], (i) X-ray crystallographic techniques: Here structures of intermediates of ATPase cycle is studied by using analogues which trap the protein at different points in the cycle. (ii)Experiments which use optical trapping, atomic force microscopy or total internal reflectance fluorescence microscopy: Here release of molecules such as ATP can be monitored by the fluorescence and the corresponding mechanical movement can be inferred.(iii) Site specific mutation studies which can give information about which domains of the protein are involved in force generation. (iv)Optical tweezers are used to manipulate the motors and observe how they respond to an opposing force. A sensitive motion detector records the movements produced with nanometer scale resolution. (v) Physical modeling of molecular motors are inspired by appearance of a new generation of experiments. Both the filaments and the motor proteins can be purified to such extent that clean reproducible experiments can be performed in vitro. These experiments can be divided in two classes: (a) the motion of single filaments propelled by many motors which are absorbed on a flat substrate is monitored optically ;or (b) the motion of single motors along filaments is observed.

In the framework of theoretical physics there are two main problems that can be addressed regarding molecular motors. Both problems fall in the realm of non equilibrium systems. The first is Mechanical transduction of energy. That is, how this molecular scale machinery behaves differently from macroscopic engines. The second is how statistical properties of matter in the cell are modified by the action of motor proteins. This includes the important issues such as self-organization of the cyclo skeleton and mitosis.In the second kind of problems the emphasis is on how the self organization of microtubules is affected by the motor driving[?]

The early modeling of muscle contraction assumed the existence of several states of a motor, within each of which the system reaches local thermodynamic equilibrium on time scales which are small compared to the exchange rates between these states. From the transient response of the muscles it was known that the fastest characteristic times of the motors is in the range of milli seconds. Thermal equilibrium occurs on length scales of 10nm after tens to at most hundreds of nano seconds. The states of proteins during muscular contraction therefore had to be in local thermodynamic equilibrium. To explain the mechanism of force generation, asymmetry(polarity of the motor or filament interaction)and the importance of chemical energy consumption were needed. The asymmetry of the system was introduced via asymmetric transition rates. [?]

The in vitro experiments raise the question of the interference of fluctuations and Brownian motion with the directed motion that is characteristic for these motors. A simple example for the modeling of such a process is a generalization of Feynman's famous thermal ratchet which shows that a periodic distribution of temperatures with the proper asymmetry was sufficient to induce macroscopic motion of a particle in a periodic potential via a rectification mechanism of the random Brownian forces. Since for molecular motors any temperature inhomogeneity at the scale of a few tens of nanometers decays on timescales of microseconds, the ratchet mechanism cannot be applied directly for describing motors at the nanometer scale.

The various mechanisms proposed to explain the molecular motors which work in the nonlinear regimes far from equilibrium are[?]

(i) Fluctuating forces: a point-like particle is placed in a periodic, asymmetric potential $W(x)$

and is submitted to a fluctuating force which does not satisfy a fluctuation dissipation theorem. Typically the particle motion is described by the Langevin Equation $\xi\frac{dx}{dt} = -\partial_x W(x) + F(t)$, where ξ is the constant friction coefficient, x the position of the particle and $W(x)$ is the potential energy which it experiences. The fluctuating force $F(t)$ has zero averaged value $< F(t) >=0$ but has richer correlation functions than a simple Gaussian white noise. These correlations of the fluctuating forces reflect the energy source: their structure depends on the complexity of the underlying chemical process. As soon as the fluctuation-dissipation theorem is broken, a rectified motion sets in with a direction that depends in a subtle manner on the details of the statistics.

(ii) Fluctuating potentials: A point-like particle is placed in a periodic, asymmetric potential with a value that depends on time: $\xi\frac{dx}{dt} = -\partial_x W(x,t) + f(t)$, with a time dependent potential $W(x,t)$ and $f(t)$ is Gaussian white noise which obey the fluctuation-dissipation theorem:$< f(t) >= 0; < f(t)f(t') >= 2\xi T\delta(t-t')$ The energy source is now implicit on the time dependence of the potential W.

(iii)) Particle fluctuating between states: Here, the notion of well defined states is used. In each of these states, the "particle" experiences a classical Langevin equation $\xi_i\frac{dx}{dt} = -\partial_x W_i(x,t) + f_i(t)$, with i referring to the state and $f_i(t)$ satisfies the fluctuation dissipation theorem $< f_i(t) >= 0; < f_i(t)f_j(t') >= 2\xi_i T\delta(t-t')\delta_{ij}$. Here the rectification is obtained if at lest one of the transition between states does not satisfy detailed balance.

3 Results and Discussion

Micro mechanical measurements and modeling may be combined with biochemical and structural studies to provide a view of how protein motors may generate force. Biochemical studies imply existence of some form of mechanical signal that inform one motor head that ATP has bound to the other, thereby triggering ADP release from the former. Structural studies have shown that, in the absence of ATP, the neck linker region exists in multiple configurations, and that binding of ATP serves to stabilize this domain, causing it to associate more tightly with the motor core. All these studies suggest that the neck of the motor serves a critical mechanical amplifier. The directionality is also probably controlled by the neck region. It is found that the motor domain of the kinesin-related protein Ncd moves in the opposite direction to kinesin, even though the two motors display a high degree of the amino acid sequence identity. Ncd has a similar core domain as kinesin but a different neck region. It is possibly the different conformational changes happening in the neck region that is driving the two in opposite directions. This is further confirmed by another interesting experimental observation that by mutation of a single amino acid in the neck region of Ncd protein changes its direction of motion[?]. It is also found that in its ATP driven movement along a microtubule, conventional kinesin can take over a hundred steps without dissociating. The two heads of the kinesin allows this. The two heads bind alternatively while walking on the microtubule. It is also shown that a single kinesin molecule suffices to move a microtubule through distances of several microns. The microtubule is moved at a rate of one micron per second. By tethering single molecules of kinesin to tiny tensiometers- a flexible glass rod or an optical trap- it is possible to measure the force exerted by a single kinesin molecule. The maximum force is found to be approximately 5 pN. These experiments show that the kinesin can do mechanical work of 4

10^{-20} J of work per step, and if there is just one ATP consumed per step, then the efficiency is 40%.

To make the analysis analytically tractable in most of the physical approaches two state models are used,the idea being that it is enough to give physical insight into many physical realizations. These models show the importance of time scales involved as well as the spatial dependence of chemical activity. If collection of such motors are considered, dynamical phase transitions similar to the liquid-vapor transition is also observed which implies the possibility of spontaneous oscillations whenever elastic elements are added in series to the motor collection.

In the study of Biological problems, the approach to the problem differs in details, methods and goals beyond understanding between physicists and biologists. The biologists in this case are more interested in studying the conformational changes happening during a cycle of motor operation. For this purpose they are using increasingly sophisticated experimental techniques. Till now it cannot be said that the specific conformational changes have been identified without doubt. But the fact that it is conformational changes that is driving the motors as well as responsible for force production has been established without doubt. Increasingly sophisticated experimental techniques and bio-chemical reasoning is hoped to make the understanding of protein motors concrete in the future. It is suggested that better understanding of motor activity will aid us in understanding certain diseases (like Down Syndrome) whose origin is thought to be improper cell division due to the malfunction of motor proteins. Another biological benefit suggested is the use of motor proteins as directed carriers of drugs in the drug delivery problem.

Physicists on the other hand are trying to understand the main physical mechanism responsible for motor motion in the context of established physical reasoning which is mostly derived from non-equilibrium thermodynamics. The ratchet mechanism of rectification of Brownian noise is accepted as the driving force. Here the model used to describe the motor is very simplistic devoid of any structural details nor is this explanation specific to biological motors. It is also suggested that none of their predictions is directly testable by experiments performed on molecular motors in cellular conditions. The possible testable experiments are in vitro experiments, but here also simple ratchet mechanisms cannot give any testable predictions. The testable theory uses the approach of proposing a kinetic model with the intermediate states chosen in such a way to match the measured properties such as the velocity data at various load ranges and ATP levels.[?] These kinds of models are also useful to biologists in giving a crude estimate of possible conformational states involved in a single cycle and some details about their physical nature. It is suggested that understanding of the operation of these mesoscopic motors will assist us in developing synthetic motors which could assist us in assembling complex structures by manipulating individual atoms, with possible applications in semi-conductor industry. An example is the fact that study of the molecular motors and the ratchet mechanism responsible for their activity has inspired chemists to design synthetic molecular motors based on chemical changes induced by external stimuli driving mechanical motion.[?] Another application is in particle seperation in a mixture if we understand the contribution of external potential to the determination of direction of motion of these motors[?]

It seems that in the immediate future major contributions to the understanding of motor proteins and their function will come from the application of sophisticated experimental techniques. Nevertheless it seems that the simulation of such a system keeping details at least at the secondary structure level, which is seen to be very similar in various motor proteins, is sure to provide under-

standing beyond the bio-chemical understanding of such a system. It will be really interesting to see how the amplification of minute motions induced by ATP hydrolysis takes place over the structure and its transmission from one point in the structure to the other. This level of understanding will certainly help us in creating artificial motor proteins designed to perform a certain function. The challenges of such a simulations are, identification of rigid coarse-gained domains, effective interactions between them, and the fact that here simulations have to connect hugely differing time scales(from pico seconds to milli second).

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